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A CONSIDERATION OF ARSPHENAMINE AND CERTAIN OTHER ORGANIC ARSENIC COMPOUNDS USED IN THE TREATMENT OF SYPHILIS.[1]

By GEORGE B. ROTH, M. D., Pharmacologist, Hygienic Laboratory, United States Public Health Service, Washington, D. C.

The organic arsenic compound arsphenamine, formerly known as "salvarsan," which is used so extensively in the treatment of syphilis to-day, was discovered by Ehrlich and Bertheim in 1910, after many painstaking and systematic researches, it being the 606th compound which they synthesized. The advent of arsphenamine into therapeutics, therefore, was in no way accidental, but was the result of methodical chemical and biological study.

The treatment of syphilis by means of arsenic was not new when arsphenamine was originated, arsenic in the inorganic form having been used intermittently since the time of Fallopius. In order to comprehend clearly the nature of the better known antisyphilitic agents containing organic arsenic as their main active constituent, I shall briefly sketch their chemical relationships without attempting, however, to arrange the compounds with any strict reference to the chronological order of their introduction.

Leaving out of consideration, therefore, the inorganic compounds of arsenic represented by the highly toxic trivalent compounds of arsenious acid and the less toxic pentavalent compounds of arsenic acid, we can satisfactorily arrange most of the antisyphilitic agents containing arsenic as their main active constituent into three groups, namely, (*a*) the cacodylates, (*b*) the arsanilates, and (*c*) the arsenobenzols, all of which contain arsenic in the organic form.

(*a*) *Cacodylates.*—The cacodylates are aliphatic compounds containing arsenic in the pentavalent form and are close derivatives of arsenic acid.

By substituting two methyl groups for two hydroxyl groups in arsenic acid, cacodylic acid is formed; and by further substitution of sodium for the hydrogen of the remaining hydroxyl group, one obtains sodium cacodylate.

Another closely related salt of cacodylic acid, which differs from sodium cacodylate in having one of its methyl groups replaced by an NaO group, was introduced into therapeutics as "arrhenal."

Mention should perhaps be made at this point of the recently introduced homologue of cacodylic acid, namely, disodium ethyl arsonate, which appears under the trade name of "mon-arsone." This homologue differs from "arrhenal" in having the methyl group of the latter substance replaced by an ethyl group.

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[1] Address delivered at the quarterly conference of clinic directors and physicians, Boston, Mass., Apr. 13, 1921.

The relation of these compounds to arsenic acid and to each other may be seen from the following structural formulæ:

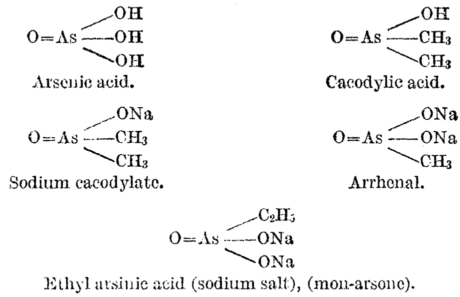

CHART 1.

The announcement by Ehrlich in 1910 that arsphenamine was curative in syphilis, led Murphy, a well-known clinician,[2] who was unable to secure Ehrlich's remedy, to try sodium cacodylate for this disease. He was surprised to find that excellent results were obtained with sodium cacodylate in healing primary chancres and certain other manifestations of syphilis. The cacodylates immediately met with favor in the United States in the treatment of syphilis, and in spite of later evidence that their popularity was unjustified, they are still used in certain quarters as antisyphilitic agents.

The effects of the cacodylates are considered to be due principally to the partial reduction of these compounds in the body; but since the reduction occurs slowly, the toxic effects are not pronounced. A large percentage of the cacodylate compounds is eliminated unchanged. The amount which may be reduced varies in different individuals and conditions, so that the cacodylates may not always act as harmless agents.

Comparative tests made with certain of the cacodylates and arsphenamine show that the cacodylates are decidedly inferior to arsphenamine, both in experimental syphilis and in human cases. As regards the homologue of cacodylic acid, disodium ethyl arsonate or "mon-arsone," little can as yet be stated with certainty. The clinical reports of its use in syphilis, though on the whole favorable to the compound, are still too meager to permit one to state positively whether it will rank with arsphenamine as an effective antisyphilitic agent; but, as in the case of the cacodylates, the results in experimental syphilis have been disappointing.

(*b*) *Arsanilates.*—The arsanilates differ from the cacodylates in being aromatic type derivatives of arsenic acid, and are made by combining arsenic acid with aniline in the presence of an alkali. They may be regarded as close chemical forerunners of arsphenamine. Although Béchamp in 1860 found that aniline and arsenic acid could be combined, it remained for Ehrlich and Bertheim in 1907 to determine the true nature of the compound and to establish it as being arsanilic acid. The sodium salt of arsanilic acid, sodium arsanilate, was introduced as an especially nontoxic agent for the treatment of syphilis under the name of "atoxyl."

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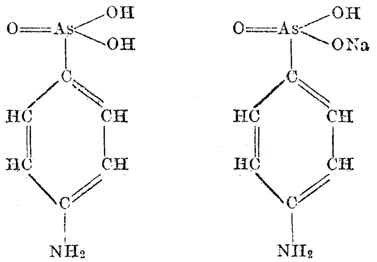
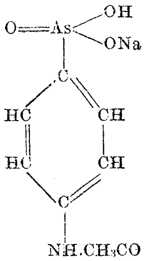
[2] Murphy, J. B. Jour. Am. Med. Assoc., 1910, vol. 55, p. 1113.

Sodium acetyl arsanilate, a compound closely allied to "atoxyl," was introduced as "arsacetin."

The structures of the above-described arsanilates are given below:



Arsanilic acid.   Sodium arsanilate (*atoxyl*).



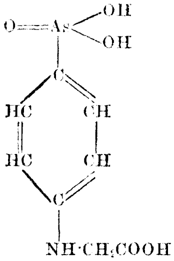
Sodium acetyl arsanilate (*arsacetin*).

CHART 2.

"Atoxyl" proved to be of considerable value in the treatment of syphilis; but, after thorough trial, it was found to be much more toxic than it was first supposed to be, causing permanent blindness in a considerable number of instances. Ehrlich found that "atoxyl" had no effect on trypanosomes outside of the body and, hence, explained its effects in syphilis by its reduction in the body, from the pentavalent to the trivalent form. The decomposition products, however, have never been isolated. It is decomposed very readily in the gastro-intestinal tract, forming more toxic products; therefore it is not given by mouth.

"Arsacetin" was found to be quite dangerous and no more effective than "atoxyl" in syphilis.

The explanation given by Ehrlich to account for the parasiticidal action of "atoxyl," namely, that it was reduced from the comparatively inactive pentavalent to the more active trivalent form, led him to study other aromatic arsenic compounds in which the arsenic is trivalent.



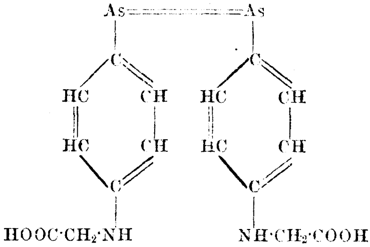
Phenyl glycine p arsinic acid.

CHART 3.

His investigation led to the discovery of phenyl glycine p arsinic acid. Chemically phenyl glycine p arsinic acid is an important derivative of arsanilic acid and is distinguished from "atoxyl" mainly by its decreased toxicity.

(*c*) *Arsenobenzols.*—Phenyl glycine p arsinic acid, on reduction with sodium hydrosulphite, yields arseno phenyl p glycine, a trivalent compound which is very important from the standpoint of therapeutics, and which represents the third or arsenobenzol type. In this type two arsenic atoms are linked together by a double bond, and each to the benzol nucleus by a single linkage.

The relation of arseno phenyl p glycine to arsphenamine is very close, as seen from its structure given below:

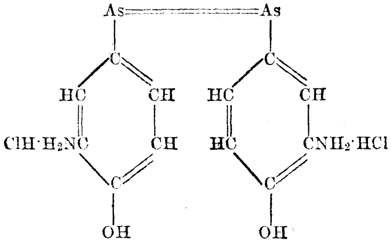


Arseno phenyl p glycine.

CHART 4.

Arseno phenyl p glycine was found by Ehrlich to have a marked influence on the course of relapsing fever in mice. Its sodium salt, sodium arseno phenyl p glycinate, 418 in Ehrlich's series, appeared under the name of "spirasyl."

By varying and changing the position and kind of sidechains, Ehrlich found that the most beneficial results were obtained, not only in mice infected with relapsing fever but in human syphilis, with the dihydrochloride of 3.3′ diamino, 4.4′ dihydroxy arsenobenzene, 606 in Ehrlich's series, or "salvarsan," now known officially as arsphenamine.

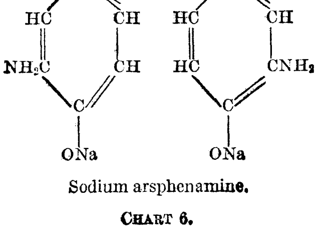


Arsphenamine.

CHART 5.

Ehrlich considered that this compound embodied the virtues which a compound that was to be used in syphilis should have, namely, a marked parasiticidal action with little or no deleterious action on the host. All previous compounds which possessed parasiticidal properties were also quite destructive to the host, so that in arsphenamine Ehrlich's desires seemed to be realized.

Aqueous solutions of arsphenamine are quite strongly acid and should not be used in therapeutics on account of their high toxicity. For this reason, arsphenamine should always be properly alkalinized before its administration. The disodium salt of arsphenamine which is thus formed upon the addition of the proper amount of sodium hydroxide, is less stable than arsphenamine and, hence, was not considered as a commercial possibility until quite recently. The commercial product now appears under the official name of sodium arsphenamine (1206 of Ehrlich's series). Although it can be prepared for administration by the simple addition of water, and in this respect is treated like neoarsphenamine, it should be administered according to the rules formulated for arsphenamine. Structurally, it is essentially the same as arsphenamine.

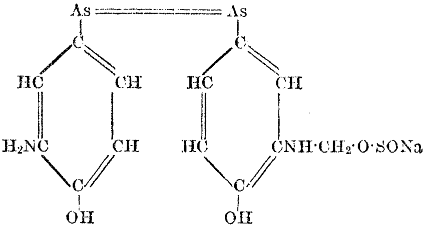


Sodium arsphenamine.

CHART 6.

The discovery that arsphenamine possessed marked parasiticidal properties led Ehrlich to the further investigation of various derivatives of arsenobenzol, and, in seeking to obtain a preparation which could be used clinically without preliminary treatment with an alkali, he discovered that by treating arsphenamine with sodium formaldehyde sulphoxylate the following condensation product was formed, namely, sodium, 3.3′-diamino-4.4′-dihydroxy-arseno-benzene-N-methylene sulphinate.

This product is known as 914 in Ehrlich's series, and, later, was given the name of "neosalvarsan." It is now known officially in the United States as neoarsphenamine. The following structure has been generally assigned to it, perhaps without sufficient evidence:

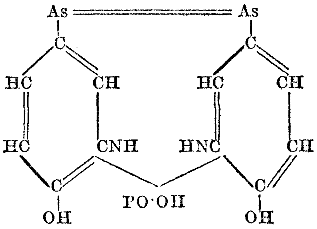


Neoarsphenamine.

CHART 7.

The curative properties of neoarsphenamine in syphilis need not be mentioned, except to say that it seems to be the leading arsenical to-day in the treatment of this disease.

Since the advent of arsphenamine and neoarsphenamine into therapeutics, a great amount of research has been directed toward improving these compounds of the arsenobenzol type, and, as a result, many new compounds have been discovered. None of these newer compounds, however, has supplanted, nor has even closely rivaled, arsephenamine and neoarsphenamine in favor. After numerous investigations the Treasury Department of the Federal Government officially recognized by license only two compounds in addition to arsphenamine, sodium arsphenamine, and neoarsphenamine. The first of these additional compounds is an arsphenamine derivative containing phosphorus, and is 4.4′ dihydroxy arsenobenzene 3.3′ phosphamic acid (No. 1116 of Mouneyrat's series). It is licensed under the official name of phospharsenamine, its trade name being "galyl." Its structure is given by Morgan,[3] as follows:

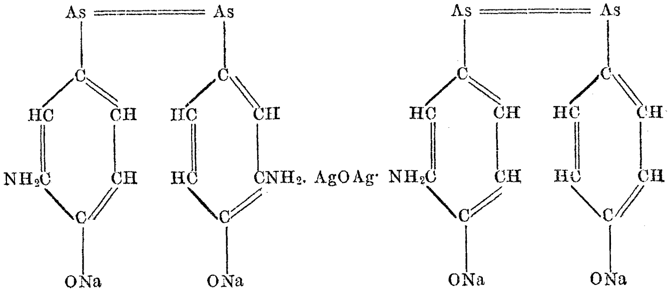


Phospharsenamine (galyl.).

CHART 8.

Phospharsenamine is an acidic substance, insoluble in water, and dissolves in aqueous sodium carbonate. It is put up in sealed ampules containing the dry sodium carbonate, so that it will readily dissolve upon the addition of water. Solutions made in this way are yellow, or greenish yellow, the greenish color rapidly deepening upon standing.

The second additional compound is an arsphenamine derivative containing silver. Its chemical composition is not definitely known. It is officially known as silver arsphenamine, and is thought to have the following structure:



Silver arsphenamine.

CHART 9.

Silver arsphenamine dissolves readily in water, forming a black or brownish-black solution. Both the phosphorus and silver compound of arsphenamine are considered to be improvements over arsphenamine; but as they have been but recently officially licensed by the Federal Government, adequate clinical reports of their use in this country are unavailable at present. Both are enjoying considerable reputation abroad.

In conjunction with the official name, arsphenamine appears in the United States under the trade names of "salvarsan," "arsenobenzol," "diarsenol," and "arsaminol"; sodium arsphenamine as "sodium diarsenol" and "salvarsan sodium"; neoarsphenamine as "neosalvarsan," "neodiarsenol," and "neoarsaminol"; silver arsphenamine as "silver salvarsan" and "silver diarsenol," and finally, phospharsenamine as "galyl."[4]

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[3] Morgan, Gilbert T., Organic Compounds of Arsenic and Antimony. 1918.

The behavior, both inside and outside of the body, of compounds of the arsenobenzol type used in the treatment of syphilis, is of extreme interest and also of great practical importance, since they are used so extensively in the disease. Unfortunately, too little is known concerning their chemical nature and their behavior in the organism. In making this last statement I do not wish to disparage the work of Ehrlich and his coworkers. Their names will undoubtedly always be associated with the greatest of epochs in the history of therapeutic agents. However, when one considers that the vast amount of work which has already been done has not given us even a chemical method for determining a toxic from a non-toxic preparation of arsphenamine, we can the more readily realize that much is still to be learned regarding this active type of compounds.

During the past few years I have had the opportunity, in the Hygienic Laboratory of the United States Public Health Service, of examining for toxicity over 2,000 samples of arsphenamine and neoarsphenamine, and in this manner I have been permitted to observe the behavior of these compounds, both *in vitro* and *in vivo*, under carefully controlled conditions. As a result of these observations certain studies were made and certain data compiled which were of value in conducting the biological tests required by the Federal Government before these compounds could be sold in interstate traffic. The clinical bearing of these studies will be obvious from the description of the several experiments which appear later in this paper.

The official method for testing arsphenamine requires that white rats weighing from 100 to 150 grams should tolerate per kilo of body weight, 100 mgm. of the drug for 48 hours when given intravenously as a 2 per cent alkaline aqueous solution, 0.9 c.c. of normal sodium hydroxide being used to alkalinize 100 mgm. of arsphenamine. Neoarsphenamine is also required to be tested in white rats, the dose to be tolerated for 7 days being 200 mgm. per kilo when given intravenously as a 4 per cent aqueous solution. The rate of injection must be 12 to 15 seconds for each 0.1 c.c. of either solution.

One of the first observations, which has been repeatedly confirmed during the past three years, is that there is a quite marked individual susceptibility in animals to both arsphenamine and neoarsphenamine. An extreme instance is illustrated by reference to some experiments on rabbits (Roth, 1918, Hyg. Lab. Bulletin No. 113). In working with a sample of arsphenamine which killed 75 per cent at 100 mgm. per kilo and 50 per cent of the rabbits at 80 mgm. per kilo within two weeks, an animal was found which tolerated 175 mgm. per kilo for one month. Quite similar results were obtained with neoarsphenamine on rats.

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[4] Solutions of each of the licensed arsphenamines, put in hermetically sealed glass ampules, appear commercially as solutions of arsphenamine.

The impression was early obtained that arsphenamine and neoarsphenamine were not as similar in action as they are usually thought to be, many clinicians even at the present time, regarding neoarsphenamine merely as arsphenamine in a form convenient for administration. The following observations demonstrate that arsphenamine and neoarsphenamine are quite unlike biologically.

As stated above, according to the official regulations, the maximal tolerated dose of arsphenamine must be 100 mgm., and for neoarsphenamine, 200 mgm. per kilo when given to white rats intravenously. These dosages are not far below the minimal lethal dose of perhaps the majority of preparations. Now, when these compounds are administered to rats in the official dosage, the time of death of the animals treated with arsphenamine is totally unlike that observed after administration of neoarsphenamine.

Statistics compiled from the above official tests showed that after administration of arsphenamine a little over 80 per cent of the rats which died within 14 days died within 24 hours after administration, and that almost 90 per cent died within 48 hours; whereas the rats which received neoarsphenamine rarely died within the first day, and the majority died after the third day. The time of death is shown in the following table:

Total number dying:	Per cent.
Within the first day	5
Within the first two days	15
Within the first three days	30
Within the first four days	60
Within the first five days (about)	75

The following additional differences were observed:

(*a*) Neoarsphenamine rarely causes immediate symptoms in rats, whereas arsphenamine usually causes pronounced depression in the standard test doses.

(*b*) Rats injected intravenously with neoarsphenamine bleed profusely, whereas those injected with arsphenamine bleed but little, thus showing a difference in the effect on the coagulability of blood *in vivo*.

(*c*) The necropsy findings in arsphenamine-treated rats usually show marked pulmonary changes and slight kidney changes, whereas those dying from neoarsphenamine show marked degenerative changes in the kidney and relatively slight pulmonary changes. These differences are so constant that one can almost always tell from the necropsy findings what substance was administered.

(*d*) Arsphenamine was found to be relatively more stable than neoarsphenamine, the latter being frequently described in the laboratory as the more "tricky" compound of the two.

It was previously stated that acid solutions of arsphenamine were observed to be much more toxic than solutions properly alkalinized, so that failure to alkalinize may give rise to very disastrous results. This was pointed out by Ehrlich in his early studies. Strange to say, there are certain clinicians who still give unalkalinized solutions of arsphenamine. Studies made on rats showed conclusively that arsphenamine in acid solution is at least two to four times as toxic as a solution made alkaline with the proper amount of sodium hydroxide. If the arsphenamine is given as a dilute acid solution, it is less toxic than when given in concentrated solution. This fact may account for the apparent nontoxicity of acid solutions reported by certain clinicians. It was further shown in experiments with white rats that a properly alkalinized solution of arsphenamine is slightly less toxic as a 0.5 per cent than as a 2 per cent aqueous alkaline solution.

In our earliest experiments at the Hygienic Laboratory the method advocated by Ehrlich was followed in alkalinizing arsphenamine; that is, a 15 per cent solution of sodium hydroxide was added to the aqueous solution of arsphenamine, drop by drop, until the precipitate which formed was redissolved. An additional drop or two of the 15 per cent sodium hydroxide solution was then added before making the alkalinized arsphenamine up to the desired concentration. Such solutions were shown to be relatively unstable in air, becoming turbid within a half hour in many cases. It has been shown by others that solutions alkalinized according to the method of Ehrlich are more prone to produce reactions clinically than those made more alkaline. By using Ehrlich's method, the monosodium salt of arsphenamine is mainly formed, whereas when using one-third more alkali than is required to clear the solutions of the precipitate which forms, the disodium salt is formed.

That a solution of arsphenamine consisting mainly of the monosodium salt is more toxic than a solution of the disodium salt has been confirmed in experiments which we have carried out on rats. The details of these experiments appear in Table I. The addition of 0.65 to 0.7 c. c. normal sodium hydroxide per 100 mgm. of arsphenamine mainly forms the monosodium salt, 0.9 c. c., the disodium salt.

TABLE I.—*The influence of the degree of alkalization of arsphenamine on toxicity in white rats when given intravenously as a 2 per cent alkaline aqueous solution. (Inadequately and adequately alkalinized solutions were injected alternately.)*[1]

Group.	Inadequate alkalization.				Adequate alkalization.			
	0.65 to 0.7 c. c. normal sodium hydroxide per 100 mgm. of arsphenamine.				0.9 c. c. normal sodium hydroxide per 100 mgm. of arsphenamine.			
	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.
AA	5	4	4	1	5	0	0	5
BB	5	[2] 5	5	0	5	2	3	2
CC	5	2	2	3	5	0	0	5
DD	5	4	4	1	5	1	1	4
EE	5	4	4	1	5	1	1	4
FF	5	5	5	0	5	3	3	2
Total	30	24	24	6	30	7	8	22
Per cent	....	80	80	20	....	22	27	73

[1] Animals received 100 mgm. per kilo.
[2] Two were pregnant.

It will be noticed that in our experiments both the monosodium and disodium salt are made with normal sodium hydroxide (about 4 per cent) instead of using the 15 per cent solution as advocated by Ehrlich. However, the underlying principle affecting the two methods is the same. In fact, many clinicians to-day rationally use various strengths of sodium hydroxide solution. They ascertain by trial that it requires a certain amount of a sodium hydroxide solution to clear a certain amount of arsphenamine solution. To the arsphenamine solution thus cleared one-third more alkali is added, thus forming the disodium salt.[5]

The quality of the sodium hydroxide used in alkalinizing arsphenamine was also found to have a distinct influence on toxicity in white rats; none but pure sodium hydroxide should be used. Unfortunately it is very difficult generally to obtain sodium hydroxide which is free from impurities. We have lately encountered a so-called "chemically pure" sodium hydroxide, which, when made up as a normal solution, deposited, after standing several days, a considerable amount of a light-brown gelatinous material. No chemical examination was made of this material, but experiments on rats showed that arsphenamine alkalinized with the bottom portion of the sodium hydroxide solution containing the deposit was considerably more toxic than a solution made alkaline with the top or clear portion of the sodium hydroxide solution. Details of the experiment appear in Table II.

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[5] The Surgeon General of the United States Public Health Service has advised the use of 0.9 c. c. of normal sodium hydroxide (approximately 4 per cent) for each 0.1 gram of drug (Reprint No. 529 from Public Health Reports), since it has been shown that this amount will form the disodium salt, regardless of the brand of commercial arsphenamine used.

TABLE II.—*The effect on toxicity of alkalinizing arsphenamine with a solution of sodium hydroxide containing sediment as shown by its intravenous administration to white rats as a 2 per cent aqueous solution.*

Sample of arsphenamine.	A. Arsphenamine alkalinized with *top* portion of *clear* sodium hydroxide solution containing no gelatinous material.[1]				B. Arsphenamine alkalinized with *bottom* portion of sodium hydroxide solution containing gelatinous material.[1]			
	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.
L and B	5	2	2	3	5	3	3	2
D R L	5	0	0	5	5	2	3	2
M	5	0	1	4	5	2	3	2
Total	15	2	3	12	15	7	9	6
Per cent	....	13	20	80	....	47	60	40

[1] A and B were made from the same sodium hydroxide solution. These arsphenamine solutions were injected alternately in each group of 10 rats, in dosage of 100 mgm. per kilo.

The influence of rate of injection on toxicity of properly alkalinized solutions of arsphenamine was ascertained in both rats and guinea pigs, and it was shown that with certain lots increasing the rate of injection increased the toxicity in both species, the effects being more marked in guinea pigs than in rats.

The effect of increasing the rate of injection in the guinea pig was shown not only by the above-mentioned increase in mortality, but by the additional observation that all guinea pigs receiving the solution rapidly were more greatly depressed immediately after the injection than those injected slowly. The results of the experiments in rats are shown in Table III.

TABLE III.—*Influence of the rate of injection of arsphenamine in white rats when given intravenously as a 2 per cent alkaline aqueous solution.* (*Alternately injected at rapid and slow rate. Animals received 100 mgm. per kilo.*)

Group.	Rapid injection.				Slow injection.			
	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.	Number injected.	Number died within 48 hours.	Number died within 14 days.	Number lived 14 days.
AX	5	4	4	1	5	1	2	3
BX	5	3	3	2	5	2	4	1
CX	5	2	3	2	5	1	1	4
DX	5	2	2	3	5	0	0	5
EX	5	2	2	3	5	3	3	2
Total	25	13	14	11	25	7	10	15
Per cent	....	52	56	44	....	28	40	60

Arsphenamine in alkaline solution is generally regarded as a very unstable substance, and the probability of a change in toxicity occurring while in solution previous to its administration must be considered in investigations dealing with the toxicity of arsphenamine. Ehrlich (Soziale Kultur und Volkswohlfahrt, 1913) found that arsphenamine in solution (presumably alkaline) increased in toxicity so rapidly that after six hours its toxicity was more than doubled, the increase in toxicity being attributed to the formation of 3-amino-4-hydroxy-phenyl-arsenoxide, or so-called "arsenoxide." However, it was shown in the Hygienic Laboratory (Roth, 1918, Hygienic Lab. Bull. No. 113) that an aqueous alkaline solution of arsphenamnie could be kept in a glass cylinder in which only a small surface was exposed to the air, for at least five hours without any increase in toxicity. Since these experiments were reported, some striking data have been collected which demonstrate clearly that alkaline solutions of arsphenamine may at first decrease slightly in toxicity on standing, provided only a small amount of surface is exposed to the air.

Tabulations made of the time of death in each set of five rats used in the official testing of all arsphenamine from three different manufacturers received during a period of one year, showed that when tested in the official manner, at the dosage of 100 mgm. per kilo, the first rat of the set was more likely to die than any of the others, and that the collective mortality was much less in the fifth rat than in the first. In other words, during the conduct of the test there was a gradual decrease in the toxicity of alkaline arsphenamine solution when exposure to the air was relatively slight, as was the case in these tests, the only surface exposed being that permitted by a 10 c. c. glass cylinder having a diameter of about 13 mm. Table IV shows the collective results in detail. Only those sets were included in which not more than 4 nor less than 1 rat died in each set. The time elapsing between the injection of the first rat and the completed injection of the fifth rat averaged about 20 minutes.

TABLE IV.—*Time of death of the rats in each set of 5 rats used in the official testing of arsphenamine. (Sets in which not less than 1 nor more than 4 died in each set.)*

Manufacturer.	Number of sets injected.	Dose in mgm. per kilo.	Total number died within 48 hours, in order of their injection.				
			1	2	3	4	5
M	41	100	34	22	17	9	13
A	55	100	25	16	17	13	10
D	34	100	19	11	5	2	5
Total	130	....	78	49	39	24	28
Per cent died, based on total number of deaths	....	....	36	22	18	11	13

It is noted that the first rat of each set of five is almost three times more likely to die than is the fifth; two times more likely to die than the third; and about 1.6 times more likely to die than the second.[6]

The shaking of aqueous alkaline solutions of arsphenamine and aqueous solutions of neoarsphenamine in the presence of air was found to materially increase the toxicity of solutions of each compound.[7] For example, shaking a 4 per cent aqueous solution of high-grade neoarsphenamine in the presence of air for one minute will convert it into a dangerous solution; that is, instead of being tolerated in rats in dosage of 420 mgm. per kilo for 7 days, as was the case when not shaken, it killed 40 per cent of the animals in dosage of 200 mgm. per kilo. If a border line preparation is used—that is, one which is tolerated by rats receiving the standard test dose of 200 mgm. per kilo but fails at a 20 per cent higher dosage—we find that shaking for 1 minute will cause it to kill every rat injected at the standard test dose.

Quite similar results were produced by shaking properly alkalinized arsphenamine solutions for a similar period of time. The increase in toxicity is presumed to be due to the formation of the more toxic oxidation product, 3-amino-4-hydroxy-phenyl-arsenoxide more commonly called "arsenoxide," since air was found to be essential to the production of increased toxicity.

The keeping qualities of market samples of arsphenamine and neoarsphenamine while in sealed ampules were found to be wholly unlike. During the last two years numerous samples of arsphenamine and neoarsphenamine were submitted by clinicians to the Laboratory for reexamination. The reexamination failed to disclose any positive evidence of deterioration in the arsphenamines submitted, whereas a large number of the neoarsphenamine samples were shown to have deteriorated while in the ampule. It was found that certain lots of neoarsphenamine changed in sealed ampules, after an indefinite period, in (1) color, (2) mobility in ampule, (3) solubility, (4) toxicity, and (5) odor.

The change in color amounted in some instances to only a slight deepening of the original golden yellow color, while in other cases a change to a brick red occurred, such as takes place when the powder is exposed to the air for a day or more. The lots which showed marked changes in color were usually in the form of a solid, and therefore not freely mobile in ampule. The change in solubility varied, as shown by their behavior when made up in 4 per cent aqueous solution, certain lots producing only a slightly turbid solution, others producing a fine flocculent, or coarse suspension. Filtration of the coarse suspension showed that only about 10 per cent of the material was soluble in water at room temperature within a half hour. This coarse suspension, as such, however, was not shown to be toxic for rats in all cases. Likewise, an increase in toxicity could not be demonstrated in each lot of neoarsphenamine which had undergone slight changes in solubility. Although we were unable to detect an increase in toxicity in certain lots of incompletely soluble or difficultly soluble neoarsphenamine, the samples submitted were reported to have produced very grave symptoms in man, and occasionally death, so that it would appear that relatively insoluble preparations should never be used clinically. These changes in neoarsphenamine are not confined to one product, but some products show them more frequently than others. Strangely, the changes in solubility do not bear any definite relation to the date of manufacture; in one instance a lot became insoluble within 48 hours after being tested at the factory. Experiments on incubating neoarsphenamine at 37° C. while in ampule, showed that solubility can be decreased in some lots by being held at 37° C. for about nine months. Such lots emit a rather strong garlicky odor on opening the ampule.

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[6] These experiments were completed before Dr. G. C. Lake of the Hygienic Laboratory had called my attention to this phenomenon.

Hunt (Jour. Am. Med. Assoc., 1921, vol. 76, p. 854) observed this phenomenon in some alkaline solutions of arsphenamine, but not in others. The tabulation above indicates that it occurs in many if not all lots representing three commercial domestic products.

[7] Reprint No. 612 from the Public Health Reports, vol. 35, No. 38, Sept. 17, 1920.

Controls kept at a temperature of 18–20° C. for a similar period were completely soluble in water when made up as a 4 per cent solution.

Summary.

The above observations on the behavior of arsphenamine and neoarsphenamine may be summarized as follows:

(1) There is a well-marked individual variation in the susceptibility of animals to both arsphenamine and neoarsphenamine.

(2) Neoarsphenamine is so unlike arsphenamine in its biological behavior that it should not be regarded as arsphenamine in a form convenient for administration.

(3) Acid solutions of arsphenamine are at least two to four times as toxic as properly alkalinized solutions, the toxicity increasing directly with the concentration.

(4) The toxicity of properly alkalinized solutions of arsphenamine is slightly less toxic as a 0.5 per cent than as a 2 per cent solution.

(5) The Ehrlich method of alkalinizing arsphenamine, in which the monosodium salt is formed, produces a more toxic solution than the present method used in the United States, in which the disodium salt is formed.

(6) The use of impure sodium hydroxide should be avoided in making arsphenamine solutions.

(7) Increasing the rate of injection of properly alkalinized arsphenamine greatly increases its toxicity.

(8) Properly alkalinized arsphenamine solutions in many cases are more highly toxic immediately after their preparation than after the lapse of about 20 minutes.

(9) Shaking alkaline aqueous solutions of arsphenamine and aqueous solutions of neoarsphenamine in the presence of air increases their toxicity markedly.

(10) Neoarsphenamine is a relatively unstable compound in sealed ampule and after an indefinite period may show changes in (1) color, (2) mobility in ampule, (3) solubility, (4) toxicity, and (5) odor.

(11) Difficultly or incompletely soluble preparations of neoarsphenamine may be highly toxic and should not be used clinically.

(12) In some cases neoarsphenamine in ampule may be rendered insoluble by incubation at 37° C. for about a year.

The above findings point out the importance of clearly distinguishing between what might be termed the "primary toxicity" of arsphenamine and allied compounds; that is, the toxicity as determined at laboratory examination, and the "secondary toxicity," or toxicity as influenced by such factors as the method of making the solution or the manner of administration.

In concluding, it should be borne in mind that the margin of safety between the therapeutic and lethal dose of arsphenamine, for example, is extremely narrow in man as compared with certain other potent agents, and that this extremely narrow margin of safety of itself makes it necessary to regard arsphenamine as a potentially dangerous therapeutic agent, even though every precaution is taken to handle it properly.

DIGEST OF COMMENTS ON THE PHARMACOPŒIA AND THE NATIONAL FORMULARY.

Review of Hygienic Laboratory Bulletin No. 129.

"Digest of Comments on the Pharmacopœia of the United States of America and on the National Formulary for the Calendar Year Ending December 31, 1919," is the title of Bulletin No. 129 issued by the Hygienic Laboratory, United States Public Health Service.

This bulletin is the fifteenth number of the series of Digest of Comments and, like the preceding numbers, it embodies a more or less complete review of the literature for the current year dealing with the items included in the two official drug standards of the United States of America, namely, the Pharmacopœia and the National Formulary. In addition to abstracts bearing directly on these two official works, comments having an indirect interest have